AMRL-TR-67-45

THE POTENTIAL HAZARD OF STAPHYLOCOCCI AND MICROCOCCI TO HUMAN SUBJECTS IN A LIFE SUPPORT SYSTEMS EVALUATOR WHILE ON A SIMULATED GT-7 MISSION

LEONARD P. LOTTER

BONNIE S. HORSTMAN

FOREWORD

This research was initiated by the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, and was accomplished by the Department of Research of the Miami Valley Hospital, Dayton, Ohio, and the Biotechnology Branch, Life Support Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. This effort was supported jointly by the USAF under Project No. 7164, "Biomedical Criteria for Aerospace Flight," Task No. 716405, "Aerospace Nutrition," and NASA Manned Spacecraft Center, Houston, Texas, under Defense Purchase Request R-85, "The Protein, Water, and Energy Requirements of Man Under Simulated Aerospace Conditions." This contract was initiated by 1st Lt John E. Vanderveen, monitored by 1st Lt Keith J. Smith, and completed by Alton E. Prince, PhD, for the USAF. Technical contract monitor for NASA was Paul A. Lachance, PhD. The research effort of the Department of Research of the Miami Valley Hospital, was accomplished under Contract AF 33 (657)- 11716. Bernard J. Katchman, PhD, and George M. Homer, PhD, were technical contract administrators, and Robert E. Zipf, MD, Director of Research, had overall contractual responsibility.

The authors wish to acknowledge the technical advice and recommendations of Edward O. Hill, PhD, Assistant Professor of Microbiology and Surgery, and Director, Research Surgical Bacteriology Laboratories, College of Medicine, University of Cincinnati. The statistical analysis of the data was carried out by Mr. Virgil Rehg, Research Associate, Ohio State University. The authors also acknowledge the invaluable assistance of Sheldon A. London, PhD, Mr. Arselus West, and Mr. Dennis Sulick of AMRL, and Mrs. Corine Gary of the Department of Research.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT

Four human male subjects participated in a 6-week simulated aerospace study and during confinement were kept under controlled metabolic conditions. During this time 28 consecutive days were spent in a Life Support Systems Evaluator. The subjects ate fresh, heat processed, freeze dehydrated, and compressed bite sized foods while exposed to simulated aerospace stress of confinement, wearing an unpressurized MA-10 pressure suit, experimental diet, and minimal personal hygienic conditions. Body and environmental areas were sampled and the catalase-positive gram-positive cocci isolated were tested for production of coagulase, deoxyribonuclease, hemolysin, gelatinase, and utilization of mannitol. The results show that there were no significant differences in frequency of occurrence of biochemical types among subjects and among environmental areas during the chamber period. There were significant differences in the frequency of occurrence of biochemical types on nose, throat, gingiva, axilla, groin, glans penis, anus, and toe. There was no buildup of biochemical types with time in any test condition. One phage type, 29/UC-18, was recovered and was passed from one subject to the environment but not to other subjects. Despite the fact that cultures tested by the coagulase plate method were shown to be false positive when tested by the coagulase tube method, in either case the frequencies of occurrence of biochemical types did not differ significantly. The same fact was observed when the deoxyribonuclease marker was used to indicate the potentially pathogenic type. The subjects remained healthy without any decrease in resistance to infection throughout all the test conditions. Those body areas most likely to harbor potentially pathogenic staphylococci are nose and groin. In the concurrent metabolic studies the physiological, biochemical, and nutritional parameters investigated were all in the normal range of clinical values. Confinement under simulated aerospace conditions for at least 28 consecutive days and conditions of minimal personal hygiene show that no unique set of circumstances are operable that would require the establishment of special biomedical criteria.

TABLE OF CONTENTS

LIST OF TABLES

SECTION I

INTRODUCTION

Biomedical criteria required to establish the necessary personal hygiene and sanitation procedures for long term flight in space are not available. Of considerable import would be the buildup of microbial populations and the development of deleterious effects on personnel as a consequence of stress induced conditions of long term space flight derived for a variety of parameters.

Several stressful factors have increased occurrence of staphylococcal pathogenicity in man and animals. Starvation, vitamin deficiencies, and protein deficient diets are examples of nutritional stresses that have predisposed man and animals to staphylococcal infection (1-4). Mice fed a protein deficient diet (5% casein) succumbed to infection by Staphylococcus aureus while those on 20% casein did not (3). The same authors (4) reported that coagulase-negative staphylococci readily infected mice fed another protein deficient diet (corn or gluten-lysine) in contrast to the casein enriched diet. These data suggest that maintenance of nutritional balances are important in the resistance of man and animals to microbial infection.

Other stresses such as burns (5), traumatic shock (6), fatigue (7), extensive body irradiation (8), hyposecretion and hypersecretion of hormones (9), and diabetes mellitus, tuberculosis, and kidney damage (7, 10, 11) have been shown to reduce resistance to microbial infection. Although any of these factors might lower the resistance of astronauts to microbial infection during prolonged space travel, those pertaining to the nutritional status are probably more germane to the problem of space travel stress.

Micrococci, S. aureus, have been reported as predominant colonizers on human skin and body surfaces and rank foremost among the potential pathogens (12). Various products or properties of S. aureus have been associated with virulence; for example, the production of coagulase, alpha-toxin and hemolysins, leukocidin, lipase, deoxyribonuclease, phosphatase, hyaluronidase, and other enzymes, and the ability to resist phagocytosis (13). Of these properties, coagulase activity has been regarded as the main determinant of staphylococcal pathogenicity (14-17).

Phage typing represents an ancillary approach in identifying potentially pathogenic staphylococci. Blair (18) claimed that only coagulase-positive staphylococci are phage typable, although 20% to 30% of these are not lysed by typing phages. Lysogeny which confers specific prophage immunity may be responsible for insensitivity

of staphylococci to these phages (19). Most nosocomial strains of staphylococci are phage sensitive and resistant to one or more antibiotics (18).

The purpose of this study was to determine the distribution of staphylococci indigenous to humans and their environment in a controlled ecological system and to ascertain if the associated biochemical markers provide reliable criteria for pathogenicity. A buildup of these organisms or their transfer among humans and their environment, or even among specific body regions, may pose a threat to the health of humans during long term space flight. Lotter, Horstman, and Rack observed that healthy human male subjects confined in a simulated aerospace environment did not become more susceptible to staphylococcal infection (20-22). Phage typing was not employed in the first study (20). In other studies (21,22) however, transfer of the organisms occurred between the environment and subjects without any buildup of the staphylococci. During confinement, the subjects were given diets of fresh foods (23), precooked freeze dehydrated foods (24), liquid foods (25), or fresh foods (26).

This report describes the results obtained from one 6-week experiment during which time 4 human male subjects were confined in a Life Support Systems Evaluator under simulated aerospace and controlled metabolic conditions. The subjects ate a diet to be used for the GT-7 mission including fresh, frozen, heat processed, freeze dehydrated, and compressed bite sized foods. The results of the basic nutritional program are reported elsewhere (27). In these studies, selected body areas and the environment were sampled by means of dry cotton swabs which were applied to appropriate culture media. Staphylococci or micrococci were isolated from the culture media and tested for their characteristic biochemical reactions. The bacterial and fungal flora excluding the Micrococcaceae were investigated as part of the overall program (28).

SECTION II

EXPERIMENTAL METHODS AND PROCEDURES

The 42-day experimental period for 4 healthy male subjects were divided into 14 days in the controlled activity facility (CAF),* 14 days in the Life Support Systems Evaluator (LSSE),* and 14 days in the CAF. Throughout the experiment all contacts with the subjects were minimized. Only personnel gowned in sterile surgical clothing were permitted to enter the CAF. Table I contains the experimental design.

The subjects were fed a 2-day cycle diet utilizing 3 nutritionally equivalent meals. These included fresh, frozen, and heat processed foods served during the first and last 6-day intervals of the experiment. The other 30 days, subjects ate freeze dehydrated and compressed bite sized foods (27). The CAF and chamber were disinfected by sponging and spraying with benzalkonium chloride (BAC) solution. Subjects were thoroughly cleansed before entering either the CAF or chamber. Sterile washcloths, towels, and pHisoHex were used to cleanse all parts of the body. The ears and nose of each subject were cleansed by sterile cotton swabs. The subjects donned sterile surgical caps, gowns, masks, and shoes for transfer in the CAF and chamber. No subject was permitted to bathe, shave, groom hair, clean or cut the nails, change or remove clothes. Wipes were used only for personal hygiene. Instead of a dentifrice, oral hygiene consisted of water. Each subject was given one 24-hour sweat test weekly while in the CAF and one 14-day test while in the chamber. The daily activity schedule as shown in table II was followed by all the subjects except as noted; while in the chamber, subjects 39 and 40 were maintained on the night shift, ate meals at 0800, 2200, and 0300, and slept from 1400 to 2200.

Body areas sampled were divided into primary regions designated areas "A", and secondary regions designated areas "B". Areas "A" included ear, nose, throat, gingiva, axilla, groin, glans penis, anus, and toe and were sampled 11 times. Areas "B" including scalp, eye, ear, arm, and umbilicus were sampled 3 times.

Fecal samples were collected 11 times. The environmental areas included bed, aft and fore table, and personal hygiene area floor. Three additional areas sampled were personal hygiene area seat, telephone mouthpiece, and refrigerator handle. Environmental areas were sampled 12 times.

---

* The controlled activity facility (CAF) and the Life Support Systems Evaluator (LSSE) at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, were used to provide a simulated space cabin environment.

TABLE I

EXPERIMENTAL DESIGN

Test days	Location	Metabolic diet	Sweat test	Microbiological sampling			
				Body areas "A"	Body areas "B"	Environment	Feces
6	CAF	Fresh food	x	x	x	x	x
8	CAF	Experimental food	x	xx		xx	xx
14	Chamber	Experimental food	x	xxxx	x	xxxx	xxxx
8	CAF	Experimental food	x	xx		xx	xx
6	CAF	Fresh food	x	xx	x	xx	xx

x = number of times area sampled during each experimental period.

TABLE II

DAILY ACTIVITY SCHEDULE

Time		Time
0600	Wake, void, body weight, oral temperature, pulse, blood pressure, BMR, blood samples when required	0600
0700		0700
0800	Eat meal A	0800
0900	Microbiological sampling when required	0900
1000	Preliminary wash or final rinse for sweat test when required	1000
1100		1100
1200	Physiological measurements, eat meal B	1200
1300		1300
1400	Preliminary wash or final rinse for sweat test when required	1400
1500		1500
1600		1600
1700		1700
1800	Physiological measurements, eat meal C	1800
1900		1900
2000		2000
2100		2100
2200		2200
2300	Physiological measurements, sleep	2300

Samples of areas "A" and "B" were collected with sterile dry cotton swabs which were streaked on 5% sheep blood agar (B.B.L.) plates. The plates were incubated aerobically at 37°C for 24 hours and then incubated at 30°C for 48 hours. The latter incubation enhanced colonial morphology and pigmentation. Fecal plates were provided by personnel from Republic Aviation Corporation. One loop of fecal material was inoculated into Gall's broth (28) and a dilution series was prepared. One-tenth of a milliliter of the 10^{-5} and 10^{-6} dilutions was plated on 10% sheep blood agar (B.B.L.) plates and incubated aerobically 37°C for 24 hours. Ten percent sheep blood agar (B.B.L.) plates were exposed for one-half hour in environmental areas including bed, fore table, aft table, and personal hygiene area floor. The personal hygiene area seat, telephone mouthpiece, and refrigerator handle were sampled by dry swabs. These swabs were put in 10 ml Trypticase Soy Broth and incubated at 37°C aerobically. After 24 hours of growth, 0.1 ml of the most suitable saline dilution of broth was coated on the surface of 5% sheep blood agar plates by a sterile glass spreader. These plates were incubated at 37°C for one day.

The replica plating technique of Lederberg and Lederberg (29) was adopted to simplify the biochemical study. Replicators slightly smaller than the standard 100 x 15 mm plastic petri dish were cast from aluminum alloy stock and covered with velveteen. The velveteen replicator was pressed against ten colonies grown on blood agar plates and then applied to the test medium surface.

The bacterial colonies on the initial 5% sheep blood agar (B.B.L.) plates were thoroughly examined for colonial morphology, pigmentation, and hemolysis. One of each colonial type observed was streaked on a plate of Trypticase Soy Broth (B.B.L.) plus 1.5% agar (Difco). Three percent H_2O_2 was applied to colonies of gram-positive cocci to detect catalase production and catalase-positive cocci were further tested for several biochemical reactions.

All cultures considered gram-positive cocci after microscopic observations and found to be catalase-positive were accepted for further biochemical studies. Before replication to biochemical test media, staphylococci were grown on 5% sheep blood agar plates and their colonies showed hemolysis after 48 hours of aerobic incubation at 37°C. Coagulase production and mannitol utilization were demonstrated on the coagulase-mannitol plate medium of Esber and Faulconer (30) to which 15% sterile citrated horse plasma (B.B.L.) (31) has been added. Deoxyribonuclease production was detected on DNAase Test Medium (B.B.L.) plates (32), and gelatinase production on Chapman-Stone Medium (B.B.L.) plates (33). Lotter and Horstman (34) found that results of the coagulase plate method (25) are unreliable after all coagulase-positive cultures had been tested by the coagulase tube method of Fisk (35). The test for free coagulase (35) was performed by combining aseptically 0.05 ml of a 24-hour TSB culture with 0.5 ml of 1-5

dilution of citrated horse plasma (B.B.L.) in a sterile 12 x 75 mm serological test tube. The tubes were incubated in a 37°C water bath Clots formed usually within 3 hours but occasionally were delayed until 18 hours. The method of Blair and Williams (36) was employed for phage typing coagulase-positive isolates. These isolates as well as host strains were grown in TSB for 6 hours at 37°C. Phage routine test dilutions were applied to the bacteria coated surface of TSA plates by sterile 2.5 cc disposable syringes.

The Communicable Disease Center, Atlanta, Georgia, supplied 22 strains of S. aureus from the International set for phage typing. These control cultures included strains 3a, 3b, 3c, 6, 7, 29, 42d, 42e, 47, 52, 52a, 53, 54, 55, 71, 75, 77, 79, 80, 91, 83a, and 187. Strain UC-18 was supplied by Dr. E. O. Hill, Surgical Bacteriology Department, Cincinnati General Hospital, Cincinnati, Ohio. Micrococcus roseus strain 516 and Sarcina lutea strain 533 were obtained from the American Type Culture Collection. These cultures were tested for production of hemolysis, coagulase, deoxyribonuclease, gelatinase, and mannitol utilization. The staphylococci were positive for each marker, although M. roseus and S. lutea were uniformly negative.

All control cultures were maintained on Brain-Heart Infusion (Difco) plus 1.5% agar slants and transferred every 2 months.

Statistical test included analysis of variance χ^2, and Student's t-test. The factors of body areas "A": subjects, time, body areas, and interaction were tested by analysis of variance at the 0.01 level of significance (37). With sampling period five omitted in each case, the first and last halves of the sampling periods were summed. Thus 2 measures for each subject and body area were obtained. To simplify statistical handling of the data for the analysis among subjects as a function of time and test conditions, the staphylococci were grouped into three catagories on the basis of biochemical reactions: CM-isolates produced coagulase and utilized mannitol; D-isolates produced deoxyribonuclease; X-isolates were positive for all except CM and Y-isolates were positive for all except D. A separate analysis was run on the CM frequencies, another on the D, another on the X (all biochemical types positive except for CM), and another on the Y (all biochemical types positive except for D).

The factors in body areas "A" were tested as follows: a test was carried out on the 4 subjects to determine if a significant difference existed among subjects. The test for time was made to determine if a significant difference in biochemical types occurred between the 2 time periods. The test for body areas was made to determine if one or more of the body areas had a significantly higher frequency than the other body areas considered. The test for interaction was made to determine the effect when 2 or more factors change at the same time. Two types of interaction considered were subject versus time and body area versus time. For example, let us

examine subject versus time interaction. If both subjects A and B possess a higher number of types by the same relative amount, no interaction can be concluded. If subject A were higher and subject B were lower, however (for the second time period), then a significant interaction would probably exist. In case of body areas "B", subjects and body areas were analyzed by a χ^2 test at the 0.01 level of significance (38). The CM, D, X, and Y frequencies were summed for each body area and subject. Time and location in the prechamber, chamber, and postchamber periods of the environment were analyzed by Student's t-test (38). This test was applied to the proportion of frequencies observed to the total possible for CM, D, X, and Y. A 0.01 level of significance was selected. The first and last halves of the sampling periods in the chamber were compared. Chamber results were matched with prechamber and postchamber results.

SECTION III

RESULTS

Tables III through V contain data obtained in this experiment. Table III demonstrates the biochemical types recovered from selected environmental areas. Areas sampled included bed, dining table, work table, and personal hygiene area floor for prechamber and postchamber sampling days in the CAF. While the subjects were confined to the chamber, the bed, fore table, aft table, and personal hygiene area floor were sampled. Potentially pathogenic staphylococci were detected by one or more of the following indices: C = coagulase production, M = mannitol utilization, D = DNAase production, G = gelatinase production; and H = hemolysis on 5% sheep blood agar. The (x) in the table indicates the occurrence of a particular biochemical type no matter how many times it was isolated. Table IV shows the biochemical types recovered from selected body areas "A" of test subjects which were, ear, nose, throat, gingiva, axilla, groin, glans penis, anus, and toe. Table V includes body areas "B" if test subjects and were scalp, eye, arm, and umbilicus.

The number of catalase-positive cocci, presumably staphylococci, totaled 882 cultures.

Table VI summarizes the results of the statistical analysis of the biochemical types recovered from the environment and selected body areas of test subjects. The frequency of biochemical types from body areas "A", body areas "B", and environment was

analyzed, respectively, by analysis of variance, χ^2, and Student's t-test. The data show that under body areas "A", the body area factor was significant for CM, D, X, and Y types. This means that in one or more body areas there occurred considerably larger frequencies of biochemical types than in other body areas (table VIII). Time was not a significant factor; the frequency of occurrence of CM, D, X, and Y types in the first 5 sampling periods did not differ markedly from the frequency of occurrence in the second 5 sampling periods. There was no buildup of any biochemical type with time. No significant difference was observed when the frequency of biochemical types was compared among subjects. Neither subject versus time nor body area versus time interactions were significant. The results indicate that the change in frequency among biochemical types from time period 1 (first 5 sampling periods) to time period 2 (second 5 sampling periods) was relatively the same for all body areas and all 4 subjects. Body areas "B" show that similar frequencies of biochemical types were isolated for all subjects and body areas. There was no buildup of CM, D, X, and Y types as the experiment progressed with time. The analysis of the environment was accomplished by making 7 separate statistical tests. When the prechamber and postchamber periods were compared, it was noted that of all the biochemical types only X was significant. The X type occurred 73% of the time in the postchamber but 20% in the prechamber. None of the types occurred more frequently from one or more areas of the prechamber, chamber, and postchamber. When the chamber and prechamber periods were compared, CM type was not significant, but D, X, and Y types were. D types occurred 2.5% of the time in the chamber and 62% in the prechamber period; X type occurred 2.5% of the time in the chamber and 20% in the prechamber; but Y types occurred 83% of the time in the chamber and 5.5% in the prechamber. When the chamber and postchamber periods were compared, CM and X type were not significant, but D and Y types were. D types occurred 2.5% of the time in the chamber and 72% of the time in the postchamber; Y types occurred 83% of the time in the chamber and 39% of the time in the postchamber. There was no apparent buildup of biochemical types in the environment as the experiment proceeded.

The coagulase reaction on the coagulase-mannitol plate medium of Esber and Faulconer (30) produced false positive results. This was shown to be the case when Lotter and Horstman (34) tested all coagulase-positive cultures (plate method) by the tube method (35). Statistical analysis was carried out on C types as determined by the tube method (35) and by the plate method (30). The results of this analysis appear in table VII which indicates statistical agreement between both methods in the frequency of occurrence of C types under body areas "A". Analysis on tube coagulase-positive types from body areas "B" and environment could not be accomplished because of insufficient data.

Table VIII shows distribution of the frequencies of biochemical types recovered from particular body areas designated as significant body areas "A" in table VI. Underlined numbers refer to those types found to be significantly higher when their averages were compared by the Duncan Multiple Range Test (37). The groin, glans penis, and anus

exhibited the largest frequency of CM type as determined by the plate method of Esber and Faulconer (30); the nose, the largest frequency of C types as determined by the tube method of Fisk (35); D types were associated most frequently in the nose and toe; X types most frequently in the nose, axilla, groin, anus, and toe; and Y types most frequently in the axilla, groin, glans penis, and anus. Of all the body areas "A" listed, the nose and groin appear as those areas most likely to carry potentially pathogenic staphylococci.

Phage typing of tube coagulase-positive isolates was employed to determine which strains of staphylococci were identical and if exchange of strains occurred between subjects and their environment. Phage type 29/UC-18 did not pass from the nose of subject 37 to the environment including bed, floor, and personal hygiene area seat until during the postchamber period. There was no instance of transmission of this phage type to other subjects.

SECTION IV

DISCUSSION

Four human male subjects were confined for a 6-week experimental period during which time they ate a diet to be used for the GT-7 mission including fresh, frozen, heat processed, freeze dehydrated, and compressed bite size foods. For 28 days the subjects lived in a simulated aerospace environment as provided by the LSSE. It is tacitly assumed that a certain degree of stress is induced by confinement, in general, by confinement in the chamber, by the experimental diet, and by the overall restrictive nature of the 6-week experimental protocol. Under the particular set of circumstances, there were no changes found in biochemical, physiological, or nutritional parameters as evaluated among the subjects (27). The data obtained in the basic nutritional study are in accord with the results obtained in the microbiological study; namely, that confinement even under minimal hygienic conditions did not cause any buildup of potentially pathogenic organisms nor did it cause lowered resistance to infection. The same results were obtained by Lotter and Horstman in studies of subjects during confinement in a simulated space chamber (20-22). The subjects ate diets of fresh foods (23) precooked freeze dehydrated foods (24), liquid foods (25), and fresh foods (26). The results agree with those of Sladen (39) who studied the effect of isolation of humans upon their bacterial flora. He found that during prolonged contact the subjects retained rather than exchanged phage types; after 12 months of isolation in the Antarctic, the total carrier rate was lowered because of the decrease

in the intermittent and occasional carrier rates. Even the persistent carriers who harbored S. aureus for as long as 2 years in the Antarctic never developed infection. It is apparent that a more definitive measure of stress, especially as related to enhancing susceptibility to infection in human subjects is needed if one is to evaluate conditions related to stressful environments.

In general, staphylococci are dispersed in the environment by air, direct contact, and contaminated objects (40). Several investigators have employed phage typing to study the modes of transmission of staphylococci in infections. While analyzing staphylococcal infections in newborns, Mortimer, et al. (41) observed that the airborne rate of transmission was 8%, whereas that by direct contact through nurses was 43%. Greendyke, et al. (42) stated that more organisms are released to the environment by fecal rather than nasal carriers. The risk of transmission of staphylococci from carriers may be considerable. In one hospital study, a single carrier infected a new patient every 14 days. If 2 carriers of a particular staphylococcus were present, a new infection occurred about every 10 days; the rate amounted to one every 7 days with 3 or more carriers (43). In the present study, only one phage type, 29/UC-18 was recovered from the nose of subject 37. Without any transmission to other subjects, subject 37 passed phage type 29/UC-18 to the environment. Perhaps nasal carriers of staphylococci should be detected and disinfected before association in a confined group..

In the present study coagulase production determined by the modified tube method of Fisk (35) was selected as the main index of staphylococcal potential pathogenicity. Lotter and Horstman (34) found that about one-tenth as many coagulase-positive cultures are detected by the tube method (35) as by the plate method (30). However, the factor(s) in the plate medium responsible for the discrepancy between the methods have not been identified.

TABLE III

RECOVERY OF BIOCHEMICAL TYPES FROM SELECTED ENVIRONMENTAL AREAS

Area sampled	Biochemical* type	Sampling day										
		Prechamber			Chamber					Postchamber		
		1	2	3	4	5	6	7	8	9	10	11
Bed	C M D G H										x	x
	C M – – H	x	x		x	x	x	x	x	x	x	x
	C M – – –		x									
	– – – G H									x	x	x
	– – – – H	x					x	x	x	x	x	
Dining table	C M – – H	x	x							x	x	x
	– – D G H										x	
	– – – G H									x		
	– – – – H			x								
Personal hygiene area floor	C M – – H	x	x	x	x	x	x	x	x		x	x
	C M – – –				x				x			
	– – – G H							x				
	– – – – H				x	x	x	x	x			
Personal hygiene area seat	C M – – H		x		x		x	x	x			
	C M – – –							x	x			
	– – – G H						x	x				
	– – – – H		x		x	x	x	x				
Telephone	C M – – H				x			x	x	x		
	– – D – H										x	
	– – – G H	x					x					
	– – – – H					x	x			x		
Work table	C M – – H	x										
	– – D – H	x										
	– – – G H	x										
Fore table	C M – – H					x	x	x	x			
	C M – – –				x							
	– – – G H				x			x				
	– – – – H					x			x			
Aft table	C M – – H					x	x	x				
	– – – – H					x	x					
Refrigerator handle	C M D G H					x						
	C M – – H					x	x	x				
	– – – G H							x	x			
	– – – – H					x	x	x				

* Biochemical type refers to those cultures with any positive reaction for the series of biochemical criteria used and are coded throughout the tables as follows: C = coagulase production; M = mannitol utilization; D = DNAase production; G = gelatinase production; H = hemolysis on 5% sheep blood agar.

TABLE IV

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "A" OF TEST SUBJECTS

Body areas	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	Postchamber 9	10	11
Subject 37												
Nose	C M D G H	x	x	x		x	x	x	x	x	x	x
	C M - - H					x						
	- - D G -				x							
	- - - G H					x						
Throat	C M D G H					x				x		
	C M - - H						x					
	- - - G H					x						
	- - - - H									x		
Axilla	C M D G H			x					x			
	C M - - H	x		x	x			x				
	- M D G H						x		x			x
	- - D G -						x					
	- - D - H					x		x		x	x	
	- - D - -		x									
	- - - G H	x	x	x		x	x	x	x	x	x	x
	- - - - H	x						x		x		x
Groin	C M D G H					x						
	C M D G -				x							
	C M D - H					x		x			x	x
	C M - - H	x	x		x		x		x			
	- - - G H		x	x		x	x	x	x	x		
Glans penis	C M D G H				x		x					
	C M D G -							x				
	C M D - H							x	x		x	x
	C M - - H	x	x	x	x	x	x			x		
	- - D G -											x
	- - - - H				x	x		x	x	x		
Anus	C M D G -								x			
	C M - - H	x	x	x								
	- - - G H	x						x		x		
	- - - - H									x		
Toe	C M D G H									x	x	
	C M D G -								x			
	C M D - H								x			
	C M - - H	x	x	x						x		
	- M D G -								x			
	- - D - H											x
	- - - G H								x	x	x	
	- - - - H	x		x	x				x	x	x	
Subject 38												
Nose	C M - - H		x									
	C M - - -					x						
	C - D G -								x			
	- - D G -				x							
	- - - G H	x	x		x	x	x	x		x	x	x
	- - - - H								x		x	
Throat	- - - G H						x		x			
	- - - - H						x					
Gingiva	C M D G H			x								
	C M - - H									x		

TABLE IV, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	Postchamber 9	10	11
					Subject 38							
Axilla	C M - - H	×										
	C M - - -								×			
	- M D G H					×		×		×		
	- - D G H										×	
	- - D G -			×								
	- - D - H			×								
	- - - G H		×	×	×	×	×	×	×			×
	- - - - H				×			×		×		
Groin	C M D G H								×	×	×	
	C M D G -				×							
	C M D - H						×					
	C M D - -				×							
	C M - G H				×							
	C M - - H			×								
	- M D G H										×	
	- - D G H					×					×	×
	- - D G -		×									
	- - - G H	×		×			×			×		
	- - - - H			×	×	×	×					
Glans penis	C M D G H									×		
	C M D - H						×					
	C M - - H	×							×		×	
	- M D G H								×			
	- M D G -									×		
	- - D G H					×						
	- - D G -				×							
	- - - - H		×			×	×					
Anus	C M D G H							×		×		
	C M - - H		×						×		×	
	- M D G H						×					
	- - D G H	×	×	×	×	×		×			×	
	- - D G -		×									
	- - - G H			×	×	×	×	×	×	×		×
	- - - - H	×		×		×						
Toe	C M - - H				×							
	- M D G H										×	
	- - D G H			×								
	- - D - H	×	×						×	×	×	×
	- - - G H	×	×	×						×	×	
	- - - - H	×	×	×	×							
					Subject 39							
Nose	C M D - H							×				
	C M - - H						×	×				
	- - D - H								×			
	- - - G H	×	×	×		×	×		×	×	×	
	- - - - H		×			×		×	×		×	
Throat	- - - G H						×		×	×	×	
Gingiva	- - - G -			×							×	
Axilla	C M D - H									×		
	C M - - H	×		×		×	×		×	×	×	

TABLE IV, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	2	3	Chamber 4	5	6	7	8	Postchamber 9	10	11
					Subject 39							
Axilla	- - - G H		×				×	×		×		
	- - - - H	×			×	×	×	×	×	×	×	×
Groin	C M - - H	×	×	×	×		×			×	×	
	- - - G H			×		×	×	×	×			
	- - - - H	×	×	×	×	×				×	×	×
Glans penis	C M - - H	×	×		×	×	×	×		×	×	
	- - - G H	×		×			×		×	×		
	- - - - H				×	×	×	×		×		×
Anus	C M D - H									×	×	
	C M - - H	×			×	×	×	×		×		×
	- - - G H		×			×						
	- - - - H			×	×	×	×	×	×	×	×	×
Toe	C M - - H	×	×	×	×				×	×	×	×
	- M D G H									×		
	- - D - H											×
	- - - G H				×					×		
					Subject 40							
Nose	C M D - -							×				
	C M - - H											×
	C M - - -										×	
	- - - G H	×	×	×	×	×	×		×	×	×	×
	- - - G -						×			×		
	- - - - H					×						
Axilla	C M - - H											×
	- - - G H	×	×	×			×	×	×	×	×	
	- - - - H	×		×	×	×	×	×		×	×	
Groin	C M D G H											×
	C M - - H	×	×	×	×	×	×		×	×	×	×
	C - - - H	×										
	- - - G H	×	×		×			×				
	- - - - H							×	×	×		
Glans penis	C M - - H	×	×			×	×	×	×		×	×
	- - - G H	×	×	×	×							
	- - - - H	×								×		
Anus	C M - - H	×	×	×	×	×		×				×
	C M - - -			×								
	- - D G H							×	×			
	- - D - H							×	×			
	- - - G H		×	×	×	×	×		×	×	×	×
	- - - - H		×			×				×		
Toe	C M D G H								×			
	C M - - H	×		×					×			
	C M - - -		×									
	- - D G H										×	
	- - D G -											×
	- - D - H				×				×		×	
	- - - G H	×	×	×	×					×		
	- - - - H	×		×						×		

TABLE V

RECOVERY OF BIOCHEMICAL TYPES FROM BODY AREAS "B" OF TEST SUBJECTS

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 37		
Scalp	C M D G H	x		
	C M D - H	x	x	x
	- - - G -			x
Eye	C M D - H		x	
	- - - G H	x	x	
Ear	C M D G H			x
	C M D G -			x
	C M D - H		x	x
	C M - - H		x	
	C M - - -	x		
Arm	C M D G H	x	x	
	C M D - H			x
	C M - - H	x	x	
	- - - G H	x	x	
Umbilicus	C M D G H		x	
	C M D - H			x
	C M - - H	x		
	C M - - -	x		
	- - D G H		x	
	- - D - H		x	
	- - - G H		x	x
		Subject 38		
Scalp	C M D - H			x
	C - D G H			x
	C M - - -	x		
Eye	- - D G H	x		
	- - D G -			x
Arm	C M - - H			x
	C M - - -	x	x	
	- - - G H		x	
Umbilicus	C M - - -	x		
	- M D G H		x	
	- - D G H			x

TABLE V, continued

Body areas	Biochemical type	Sampling day: Prechamber 1	Chamber 2	Postchamber 3
		Subject 39		
Scalp	C M D - H	x		x
	C M D - -	x		
	C M - - H			x
	- - - G H	x	x	
Eye	- - D G H	x		
	- - - G H	x		
Ear	C M D - H			x
	C M D - -		x	
	- - - G H	x	x	
Tongue	- - - - H		x	
Arm	C M - - H	x	x	
	C M - - -			x
	- - - G H		x	
	- - - - H			x
Umbilicus	C M - - H	x		
	- - - G H	x	x	x
		Subject 40		
Scalp	C M D - -		x	
	C M - - H	x		x
	- - - G H	x		x
	- - - - H		x	
Eye	C M D - -		x	
	- - - G H	x		x
	- - - G -	x		x
Ear	C M - - H	x		x
	C M - - -		x	
	- - - G H	x		
Arm	C M D G H		x	
	C M - - -			x
	- - - G H	x		
	- - - - H	x		
Umbilicus	C M D G -		x	
	C M - - H		x	x
	- - - G H			x
	- - - - H	x		

TABLE VI

SUMMARY OF STATISTICAL ANALYSIS OF BIOCHEMICAL TYPES RECOVERED FROM SELECTED BODY AREAS OF TEST SUBJECTS AND THE ENVIRONMENT

Factors	Biochemical types*		
	C, M	D	X, Y
Body areas "A"			
Body areas	S	S	S
Subjects	NS	NS	NS
Time**	NS	NS	NS
Interaction: subject vs. time	NS	NS	NS
body areas vs. time	NS	NS	NS
Body areas "B"			
Body areas	NS	NS	NS
Environment			
Prechamber vs. postchamber time	NS	NS	S, NS
Prechamber physical areas	NS	NS	NS
Postchamber physical areas	NS	NS	NS
Chamber time	NS	NS	NS
Chamber physical areas	NS	NS	NS
Chamber vs. prechamber time	NS	S	S
Chamber vs. postchamber time	NS	S	NS, S

* X = all positives except for C and M; Y = all positives except for D; S = significant; NS = not significant.

** Time period 1 compared to time period 2.

TABLE VII

SUMMARY OF STATISTICAL ANALYSIS OF BIOCHEMICAL TYPE "C" RECOVERED FROM SELECTED BODY AREAS OF TEST SUBJECTS AND THE ENVIRONMENT

Factors	C*	C**
Body areas	NS	S
Subjects	NS	NS
Time	NS	NS
Interaction: subject vs. time	NS	NS
body areas vs. time	NS	NS

* Coagulase production determined by modified tube method of Fisk (35).

** Coagulase production determined by coagulase-mannitol plate method of Esber and Faulconer (30).

TABLE VIII

FREQUENCY OF BIOCHEMICAL TYPES RECOVERED FROM SIGNIFICANT BODY AREAS

Biochemical types	Body areas*								
	Nose	Throat	Gingiva	Axilla	Groin	Glans penis	Anus	Toe	Ratio**
C, M†	15**	2	2	16	30	29	22	19	16.90
D	15	1	1	13	12	9	13	17	10.12
X	28	7	2	35	31	23	31	28	23.12
Y	27	8	3	39	34	31	35	27	25.50

* Sum of observations for all subjects in all sampling periods.

** $\text{Ratio} = \frac{\text{number of types}}{\text{sum of body areas}}$

† Coagulase production detected by coagulase-mannitol plate method of Esber and Faulconer (30).

TABLE IX

RECOVERY OF BIOCHEMICAL TYPES FROM FECES OF TEST SUBJECTS

Subject No.	Biochemical type	Sampling day										
		Prechamber			Chamber					Postchamber		
		1	2	3	4	5	6	7	8	9	10	11
37	- - - G H	x										
38	- - - G H						x					
39	C M - - -					x						
	- - - G H										x	
40	- - - G H											x

REFERENCES

1. Henius, K.: "Der einfluss des hungers und von staphylokokkenifektion auf tuberkulose kaninchen. Versuch von schlussfolgerunger auf die menschen-tuberkulose.: Bietr. z. Klin. d. Tuberk., 66: 616-630, 1927.

2. Elek, S. D.: Staphylococcus Pyogenes and Its Relation to Disease. E. and S. Livingstone, Ltd., Edinburgh, 1959, p 150.

3. Dubos, R. J., and Schaedler, R. W.: "Effect of dietary proteins and amino acids on the susceptibility of mice to bacterial infections." J. Exp. Med., 110: 921-934, 1959.

4. Dubos, R. J., and Schaedler, R. W.: "Effect of nutrition on the resistance of mice to endotoxin and on the bactericidal power of their tissues." J. Exp. Med., 110: 935-950, 1959.

5. Lowbury, E. J. L., Topley, E., and Hood, A. M.: "Chemotherapy for staphylococcus aureus in burns." Lancet, 1: 1036-1042, 1952.

6. Miles, A. A., and Niven, J. S. F.: "Enhancement of infection during shock produced by bacterial toxins and other agents." Brit. J. Exper. Path., 31: 73-95, 1950.

7. Dubos, R. J.: "The evolution of microbial diseases." Bacterial and Mycotic Infections of Man, R. J. Dubos and J. G. Hirsch (ed). J. B. Lippincott Company, Philadelphia, Pennsylvania, 1965, pp 32-34.

8. Fishman, M., and Shechmeister, J. L.: "The effect of ionizing radiation on phagocytosis and the bactericidal power of the blood. II. The effect of radiation on ingestion and digestion of bacteria." J. Exp. Med., 101: 275-290, 1955.

9. Reichlin, S., and Glaser, R. J.: "Thyroid function in experimental streptococcal pneumonia in the rat." J. Exp. Med., 107: 219-236, 1958.

10. Einaudi, M.: "Ricerche sulla localizzazione elettiva dello stafilococco piogeno aureo dopo passaggi in vitro ed in vivo su pelle." Gior. di Batteriol. e Immunol., 10: 957-980, 1933.

11. Jona, A.: "Le iniezioni di latte e le reasioni immunitarie nell' infezione sperimentale da stafilococco nel coniglio." Gior. di Batteriol. e Immunol., 9: 481-526, 1932.

12. Rosebury, T.: Microorganisms Indigenous to Man. McGraw-Hill Book Company, New York, 1962, pp 14-25.

13. Morse, S. I.: "Staphylococci and other micrococci." Bacterial and Mycotic Infections of Man, R. J. Dubos and J. G. Hirsch (ed). J. B. Lippincott Company, Philadelphia, Pennsylvania, 1965, pp 412-439.

14. Blair, J. E.: "Factors determining the pathogenicity of staphylococci." Ann. Rev. Microbiol., 12: 491-506, 1958.

15. Blair, J. E.: "What is a staphylococcus?" Bact. Rev., 26: 375-381, 1962.

16. Noble, W. C.: "Virulence and the biochemical characters of staphylococci." J. Path. Bact., 91: 181-193, 1966.

17. Panos, C., and Ajl, S. J.: "Metabolism of microorganisms as related to their pathogenicity." Ann. Rev. Microbiol., 17: 297-328, 1963.

18. Blair, J. E.: "Epidemiological implications of staphylococcal phase typing." Ann. N. Y. Acad. Sci., 65: 152-160, 1956.

19. Blair, J. E., and Carr, M.: "Lysogeny in staphylococci." J. Bact., 82: 984-993, 1961.

20. Lotter, L. P., Horstman, B. S., and Rack, J. V.: The potential hazard of staphylococci and micrococci to human subjects in a life support systems evaluator and on a diet of precooked freeze dehydrated foods. AMRL-TR-67-18, Wright-Patterson Air Force Base, Ohio.

21. Lotter, L. P., Horstman, B. S., and Rack, J. V.: The potential hazard of staphylococci and micrococci to human subjects in a life support systems evaluator and on a diet of liquid foods. AMRL-TR-67-21, Wright-Patterson Air Force Base, Ohio.

22. Lotter, L. P., and Horstman, B. S.: The potential hazard of staphylococci and micrococci to human subjects in a life support systems evaluator with elevated cabin temperature. AMRL-TR-67-43, Wright-Patterson Air Force Base, Ohio.

23. Katchman, B. J., Homer, G. M., Blanchard, W. W., and Dunco, D. P.: Biochemical and physiological evaluation of human subjects in a life support systems evaluator. AMRL-TR-66-159, Wright-Patterson Air Force Base, Ohio, February 1967.

24. Katchman, B. J., Homer, G. M., Murphy, J. P. F., and Dunco, D. P.: The biochemical, physiological, and metabolic evaluation of human subjects in a life support systems evaluator and on a diet of precooked freeze dehydrated foods. AMRL-TR-67-12, Wright-Patterson Air Force Base, Ohio.

25. Katchman, B. J., Homer, G. M., Murphy, J. P. F., Linder, C. A., and Must, V. R.: The biochemical, physiological, and metabolic evaluation of human subjects in a life support systems evaluator and on a liquid food diet. AMRL-TR-67-72, Wright-Patterson Air Force Base, Ohio.

26. Katchman, B. J., Murphy, J. P. F., Linder, C. A., and Must, V. R.: The effect of cabin temperature on the nutritional, biochemical, and physiological parameters of human subjects in a life support systems evaluator. AMRL-TR-67-107, Wright-Patterson Air Force Base, Ohio.

27. Katchman, B. J., Murphy, J. P. F., Linder, C. A., and Must, V. R.: The biochemical, physiological, and metabolic evaluation of human subjects during a simulated GT-7 mission. AMRL-TR-67-165, Wright-Patterson Air Force Base, Ohio.

28. Riely, P. E., Geib, D., and Shorenstein, D.: Determination of indigenous microflora of men in controlled environments. AMRL-TR-66-33, Wright-Patterson Air Force Base, Ohio, April 1966.

29. Lederberg, J., and Lederberg, E. M.: "Replica plating and indirect selection of bacterial mutants." J. Bact., 63: 399-406, 1952.

30. Esber, R., and Faulconer, R. J.: "A medium for initial visual demonstration of production of coagulase and fermentation of mannitol by pathogenic staphylococci." Am. J. Clin. Path., 32: 192-194, 1959.

31. Vera, H. D., Mangold, C., Zeman, J., and Olitzky, I.: "Coagulase test plates for classification of staphylococci." Public Health Lab., 17(5): 101-104, 1959.

32. DiSalvo, J.: "Deoxyribonuclease and coagulase activity of micrococci." Med. Tech. Bull., 9: 191-196, 1958.

33. Chapman, G. H.: "An improved Stone medium for the isolation and testing of food-poisoning staphylococci." Food Res., 13: 100-105, 1948.

34. Lotter, L. P., and Horstman, B. S. M.: "Comparison of a tube method and a plate method for detecting coagulase production." Am. J. Clin. Path., 48: 153-155, 1967.

35. Fisk, A: "Technique of coagulase test for staphylococci." Brit. J. Exper. Path., 21: 311-314, 1940.

36. Blair, J. E., and Williams, R. E. O.: "Phage typing of staphylococci." Bull. World Health Organ., 24: 771-784, 1961.

37. Hicks, C.: Fundamental Concepts in the Design of Experiments. Holt, Rinehart, and Winston, New York, 1964, p 165.

38. Winer, B.: Statistical Principles and Experimental Design. McGraw-Hill Book Company, New York, 1962, p 629 and p 28.

39. Sladen, W. J. L.: "Staphylococci in noses and streptococci in throats of men of isolated and semi-isolated Antarctic communities." J. Hyg. Camb., 63: 105-116, 1965.

40. Barrie, D.: "Staphylococcal colonization of the rectum in the newborn." Brit Med. J., 1: 1575-1576, 1966.

41. Mortimer, E. A., Wolinsky, E., Gonzaga, A. J., and Rammelkamp, C. H.: "Role of airborne transmission in staphylococcal infections." Brit. Med. J., 1: 319-322, 1966.

42. Greendyke, R. M., Constatine, H. P., Magruder, G. B., Dean, D. C., Garder, J. H., and Morgan, H. R.: "Staphylococci in a medical ward with special reference to fecal carriers." Am. J. Clin. Path., 30: 318-322, 1958.

43. Williams, R. E. O.: "Healthy carriage of staphylococcus aureus: Its prevalence and importance." Bact. Rev., 27: 56-71, 1963.

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Department of Research Miami Valley Hospital Dayton, Ohio 45409	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

THE POTENTIAL HAZARD OF STAPHYLOCOCCI AND MICROCOCCI TO HUMAN SUBJECTS IN A LIFE SUPPORT SYSTEMS EVALUATOR WHILE ON A SIMULATED GT-7 MISSION

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, 19 September 1965 - 1 November 1965

5. AUTHOR(S) (First name, middle initial, last name)

Leonard P. Lotter
Bonnie S. Horstman

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1967	25	43

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
AF 33(657)-11716 b. PROJECT NO. 7164 c. Task No. 716405 d.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) AMRL-TR-67-45

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited. It may be released to the Clearinghouse, Department of Commerce, for sale to the general public.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Support in part by NASA Defense Purchase Request R-85.	Aerospace Medical Research Laboratories Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, Ohio 45433

13. ABSTRACT

Four human male subjects participated in a 6-week simulated aerospace study and during confinement were kept under controlled metabolic conditions. During this time 28 consecutive days were spent in a Life Support Systems Evaluator. The subjects ate fresh, heat processed, freeze dehydrated, and compressed bite sized foods while exposed to simulated aerospace stress of confinement, wearing an unpressurized pressure suit, experimental diet, and minimal personal hygienic conditions. Body and environmental areas were sampled and the catalase-positive gram-positive cocci isolated were tested for production of coagulase, deoxyribonuclease, hemolysin, gelatinase, and utilization of mannitol. The results show that there were no significant differences in frequency of occurence of biochemical types among subjects and among environmental areas during the chamber period. There were significant differences in the frequency of occurrence of biochemical types on nose, throat, gingiva, axilla, groin, glans penis, anus, and toe. There was no buildup of biochemical types with time in any test condition. One phage type, 29/UC-18, was recovered and was passed from one subject the environment but not to other subjects. In the concurrent metabolic studies the physiological, biochemical, and nutritional parameters investigated were all in the normal range of clinical values. Confinement under simulated aerospace conditions for at least 28 consecutive days and conditions of minimal personal hygiene show that no unique set of circumstances are operable that would require the establishment of special biomedical criteria.

DD FORM 1 NOV 65 1473

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Aerobes						
Anaerobes						
Bacteria						
Staphylococci						
Phage						
Phage type						
Microbiology						
Confinement						
Aerospace						